

EXHIBIT 100

Protein Kinase Activity Profiling of Postmortem Human Brain Tissue

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Key Words

Alzheimer's disease • Drug discovery • Peptide array • Protein kinase activity • Postmortem tissue

Abstract

Background: Identification of signal transduction pathways that are critically involved in Alzheimer's disease (AD) is essential for the development of disease-specific biomarkers and drug therapy. **Objective:** This study is aimed at identifying protein kinases and signaling pathways that are activated in AD pathology. **Methods:** Microarray-based kinome profiling was employed for the detection of protein kinase activity in postmortem brain tissue derived from AD and age-matched nondemented control cases. Global serine/threonine kinase activity profiles are identified applying a peptide array system consisting of 140 peptides derived from known kinase substrate sequences covalently attached to porous chips, through which a protein solution is constantly pumped up and down. Peptide phosphorylation is determined by measuring the association of a mixture of fluorescently labeled antibodies, raised against phosphoserine- or phosphothreonine-containing peptides. **Results:** Protein lysates from freshly frozen postmortem brain tissue from nondemented controls and pathologically confirmed AD cases show ATP-dependent phosphorylation of pep-

tides. In AD and control cases, peptides that are differentially phosphorylated are identified. **Conclusion:** Protein kinase activity profiling can be used to reveal novel kinases and new signaling pathways involved in AD pathology.

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that affects a significant proportion of our aging population. Pathological features of AD include neuritic amyloid plaques, neurofibrillary tangles, neuronal loss, loss of synapses and gliosis. Most investigations have focused on the etiology and effects of neuritic plaques and fibrillary tangles, the two neuropathological hallmarks of AD. Progress in understanding the cell biological processes that lead to these neuropathological markers has provided strategies for treatment of AD. Protein kinases form a large group of regulatory enzymes that affect the biological activity of other proteins. The human genome contains about 500 protein kinase genes and kinases are thought to modify up to 30% of all proteins by transferring a phosphate group to a side chain of a specific amino acid. This phosphorylation serves as an important regulatory mechanism as it often results in conformational

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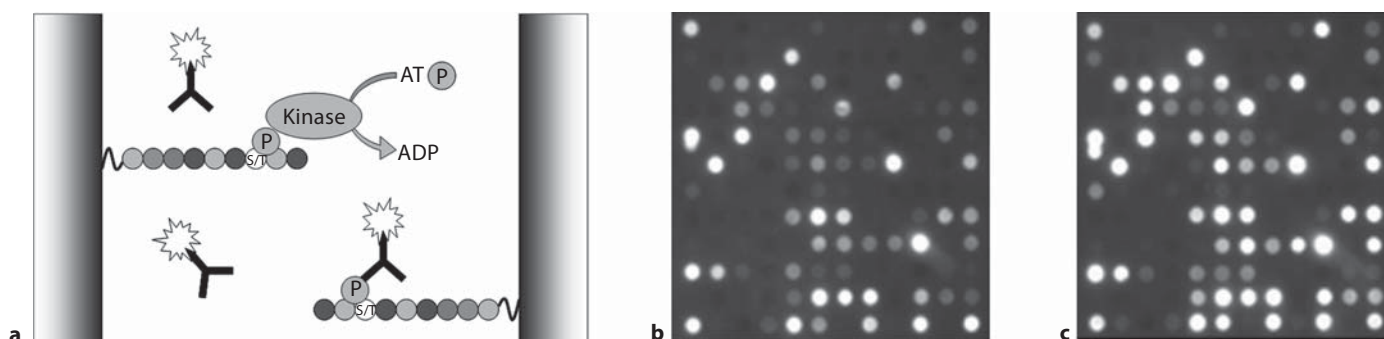


Fig. 1. a Principle of the array technology. Phosphorylation of peptides by protein kinases and detection of phosphorylated sites using fluorescently labeled antibodies against different phosphorylated epitopes. The array system is comprised of 140 peptides derived from known kinase substrate sequences and

covalently coupled to a porous carrier, allowing the protein sample to be pumped up and down, for optimizing reaction kinetics. Serine/threonine kinase activity profile of a protein lysate derived from a nonneurological control (**b**) and from an AD case (**c**).

changes in the target protein thereby altering its activity and the interaction with other proteins. Phosphorylation of target proteins is a reversible process, and proteins can be dephosphorylated by protein phosphatases. These two groups of enzymes often work together to 'turn on' and 'turn off' pathways within cells. As a result of their activity, kinases regulate the majority of the cellular pathways, especially those involved in signal transduction including those that control cell growth, cell division, apoptosis and cell death. Disrupted signal transduction pathways are involved in the etiology of many diseases. The increasing knowledge of the role of kinases in human diseases, and the growing development and availability of selective kinase inhibitors increase the interest in kinase inhibitors for drug-based therapy. Currently, protein kinase inhibitors make up about 50% of the drug discovery efforts in many large pharmaceutical companies.

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A selective number of protein kinases has been studied and their therapeutic potential has been addressed [1–4]. In most of these studies, the involvement of kinases in AD was shown using either in vitro or in vivo models for AD, or by examining protein levels in postmortem human brain tissue. Over the last few years, microarray-based kinome profiling has been developed for identifying signaling pathways in a variety of models and diseases. In this study, we have employed this technology to detect protein kinase activity in postmortem brain tissue

derived from AD and age-matched nondemented control cases. Using a flow-through peptide microarray system, we analyzed kinase activity in freshly frozen postmortem brain tissue derived from nondemented control and AD cases. The array system used for this study consisted of 140 peptides derived from known kinase substrate sequences, which had been covalently attached to porous chips (PamChip®) [5]. With as little as 0.5 µg of protein per array, protein kinase activity could be determined (fig. 1a). Subsequent data analysis revealed single epitopes showing increased or decreased ATP-dependent phosphorylation. When phosphorylation profiles of protein samples of AD and nondemented control cases were compared, significant differences were found (fig. 1b, c). Samples obtained from brain tissue with different postmortem times (varied from 6 to 8 h) gave comparable results. In addition, serine/threonine kinase activity profiles of AD and control brain tissue can be analyzed using available knowledge of substrate phosphorylation profiles of human recombinant protein kinases.

Conclusion

The assay system used in this study is unique in that it measures protein kinase activity rather than kinase expression levels and generates phosphopeptide profiles without prior hypotheses. The detection of kinase activity in lysates of cells or brain tissue, rather than the presence of a kinase, allows for a more direct relation to the events that occur in the cell or tissue. Using pathway analysis and knowledge of kinase substrate phosphorylation

patterns, the technique not only helps in the identification of active kinases, but also in elucidating differences in protein kinase activity between different samples, and in monitoring the effects of potential drug compounds on the kinase activity.

Acknowledgement

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